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Communications to the Editor

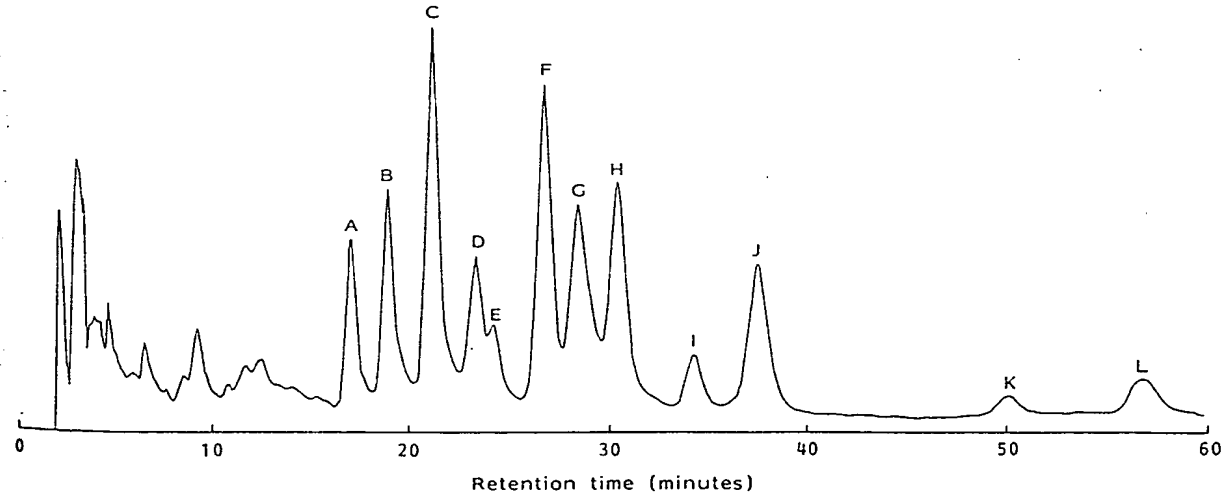
LIPOSIDOMYCINS: NOVEL NUCLEOSIDE ANTIBIOTICS WHICH INHIBIT BACTERIAL PEPTIDOGLYCAN SYNTHESIS

Sir:

The polyoxins[1] represent a class of uracil nucleoside antibiotics which inhibit chitin synthetase of various origins competitively with a substrate UDP-*N*-acetylglucosamine. Later on, tunicamycins[2] were found to be uracil nucleoside antibiotics which mimic the reaction intermediate between UDP-*N*-acetylglucosamine and lipid phosphate thus inhibiting the formation of the lipid intermediate in glycoconjugate biosynthesis of various procaryotic and eucaryotic cells. We now report a novel class of uracil nucleoside antibiotics which selectively inhibit bacterial peptidoglycan synthesis. The antibiotics have been designated as liposidomycins. At least twelve active components were observed by HPLC analysis and we have succeeded in isolation of components A, B and C in pure form.

The antibiotics are produced by an actinomycete isolated from a soil sample collected in Misaka, Yamanashi-ken, Japan. Taxonomic studies have shown that it belongs to *Streptomyces griseosporeus*. The strain was cultured at 28°C for 68 hours in jar fermentors containing a medium consisting of glucose 2%, soluble starch 1%, meat extract 0.1%, dried yeast 0.4%, soybean flour 2.5%, sodium chloride 0.2% and dipotassium hydrogen phosphate 0.005%. The culture broth (106 liters) was filtered and the mycelial cake was extracted with 80% aq acetone. After removal of acetone, the aqueous extract was combined with the filtrate and the mixed solution was adjusted to pH 7 with HCl and passed through a column of Diaion HP-10 (7 liters). The column was washed with H_2O and 30% aq MeOH, and the antibiotics were then eluted with 50% aq acetone. After removal of acetone, the resulting solution was extracted with BuOH (3 times). The organic layer was evaporated *in vacuo* and finally lyophilized, giving 28 g of a crude powder. It was purified by silica gel chromatography ($CHCl_3$ - MeOH, 1 : 1→1 : 3), and MCI-GEL chromatography (10% aq acetone→50% aq acetone). Active fractions were combined and concentrated *in vacuo* and finally lyophilized giving 2.5 g of a powder. After removal of water-insoluble matter, it was further purified by Dowex-50W (0.1 M pyridine-acetic acid, pH 4.0) and Sephadex LH-20 column chromatography (30% aq MeOH). Active fractions were combined and concd *in vacuo*. On lyophilization, the antibiotic complex was obtained as colorless powder (320 mg). By HPLC

Fig. 1. HPLC profile of liposidomycin complex.
Column: Nucleosil $5C_{18}$ ($6\phi \times 250$ mm). Solvent system: acetonitrile - buffer (0.1% diethylamine - formic acid, pH 4), 40: 60. Detector: UV (254 nm). Flow rate: 2 ml/minute.



EXH C

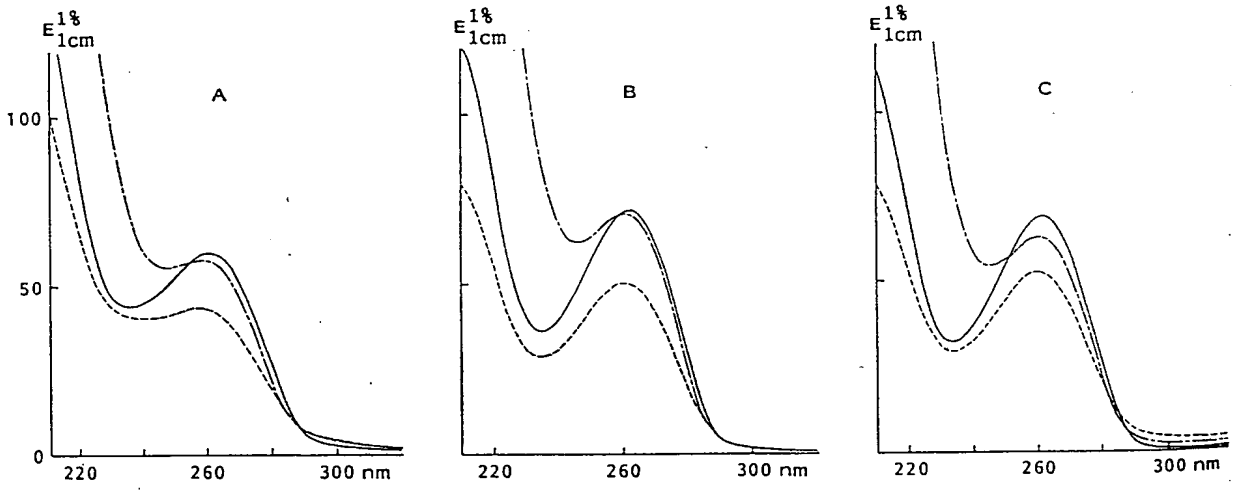
analysis (Nucleosil $5C_{18}$, 0.1% diethylamine-formic acid, pH 4 - acetonitrile, 60: 40), the complex showed twelve components (Fig. 1). Peaks A, B and C were separated by the repeated preparative HPLC. Each fraction, after removal of acetonitrile, was passed through a column of MCI-GEL. The column was washed with H_2O and then eluted with 50% aq acetone. The eluate was concd to a small volume and lyophilized. Thus liposidomycins A, B and C were obtained as homogeneous powders (yields, 2 mg, 8 mg, 12 mg, respectively).

Liposidomycin A is a colorless powder with mp >190°C (dec) and is optically active, $[\alpha]_D^{24}$ +18.7° (*c* 0.3, H_2O). The molecular formula was determined to be $C_{44}H_{67}N_5O_{21}S$ on the basis of FAB mass spectrometry [$(M+H)^+$ *m/z* 1,034, $(M+Na)^+$ *m/z* 1,056, $(M+K)^+$ *m/z* 1,072] and elemental analysis. *Anal* Calcd for $C_{44}H_{67}N_5O_{21}S$: C 51.11, H 6.49, N 6.78, S 3.10. Found: C 51.13, H 6.59, N 5.97, S 2.80. The UV spectrum (Fig. 2) showed a maximum at 260 nm ($E_{1cm}^{1\%}$ 60) in H_2O, at 258 nm ($E_{1cm}^{1\%}$ 44) in 0.1 N HCl and at 259 nm ($E_{1cm}^{1\%}$ 58) in 0.1 N NaOH.

Liposidomycin B is also a colorless powder with mp >190°C (dec) and an optical rotation $[\alpha]_D^{24}$ +17.3° (*c* 0.4, H_2O). The molecular formula was determined to be $C_{42}H_{67}N_5O_{21}S$ on the basis of FAB mass spectrometry [$(M+H)^+$ *m/z* 1,010, $(M+Na)^+$ *m/z* 1,032, $(M+K)^+$ *m/z* 1,048] and elemental analysis. *Anal* Calcd for $C_{42}H_{67}N_5O_{21}S$: C 49.95, H 6.64, N 6.94, S 3.17. Found: C 49.31, H 6.53, N 6.61, S 2.72. Two carbon (24.000 mass unit) difference between liposidomycins A and B is implied by exact mass differences measured by high resolution mass spectrometry. The UV spectrum (Fig. 2) showed a maximum at 262 nm ($E_{1cm}^{1\%}$ 72) in H_2O, at 260 nm ($E_{1cm}^{1\%}$ 51) in 0.1 N HCl and at 261 nm ($E_{1cm}^{1\%}$ 71) in 0.1 N NaOH.

Liposidomycin C is a colorless powder with mp >190°C (dec) and optically active, $[\alpha]_D$ +18.9° (*c* 0.9, H_2O). The molecular formula was determined to be $C_{42}H_{67}N_5O_{21}S$, which is isomeric to liposidomycin B on the basis of FAB mass spectrometry [$(M+H)^+$ *m/z* 1,010, $(M+Na)^+$ *m/z* 1,032, $(M+K)^+$ *m/z* 1,048] and elemental analysis. *Anal* Calcd for $C_{42}H_{67}N_5O_{21}S$: C 49.95, H 6.64, N 6.94, S 3.17. Found: C 49.52, H 6.62, N 6.63, S 2.90. The FAB mass spectrum and UV spectrum [262 nm ($E_{1cm}^{1\%}$ 69) in H_2O, 260 nm ($E_{1cm}^{1\%}$ 51) in 0.1 N HCl, 261 nm ($E_{1cm}^{1\%}$ 62) in 0.1 N NaOH] are essentially same as liposidomycin B. IR spectra (KBr) of liposidomycins A, B and C were indistinguishable from each other. Main absorption bands occur at the following approximate wavelengths: 3450, 1735, 1700, 1640, 1460, 1270, 820 and 580 cm^{-1}. They are soluble in H_2O and dimethyl sulfoxide, slightly soluble in MeOH, EtOH and BuOH, but insoluble in most other organic solvents. They gave positive reactions with potassium permanganate, anisaldehyde-sulfuric acid, anthrone and ninhydrin reagents. Acidic nature of the antibiotics was shown by the migration

Fig. 2. UV spectra of liposidomycins A, B and C.



an anode in paper electrophoresis at pH 8 (0.1 M Tris-HCl buffer). Basicity could not be detected because of instability in an acidic buffer. However, a positive ninhydrin reaction suggests the presence of an amino group.

^{1}H NMR in CD_3OD is shown in Fig. 3. The presence of uracil in components A, B and C was indicated by a pair of signals at 7.82 ppm (1H, d, J=8.0 Hz) and 5.76 ppm (1H, d, J=8.0 Hz). However, the alkyl region (0.8~1.4 ppm) is con-

Fig. 3. ^{1}H NMR spectra of liposidomycins A, B and C (400 MHz, CD_3OD, WEFT).

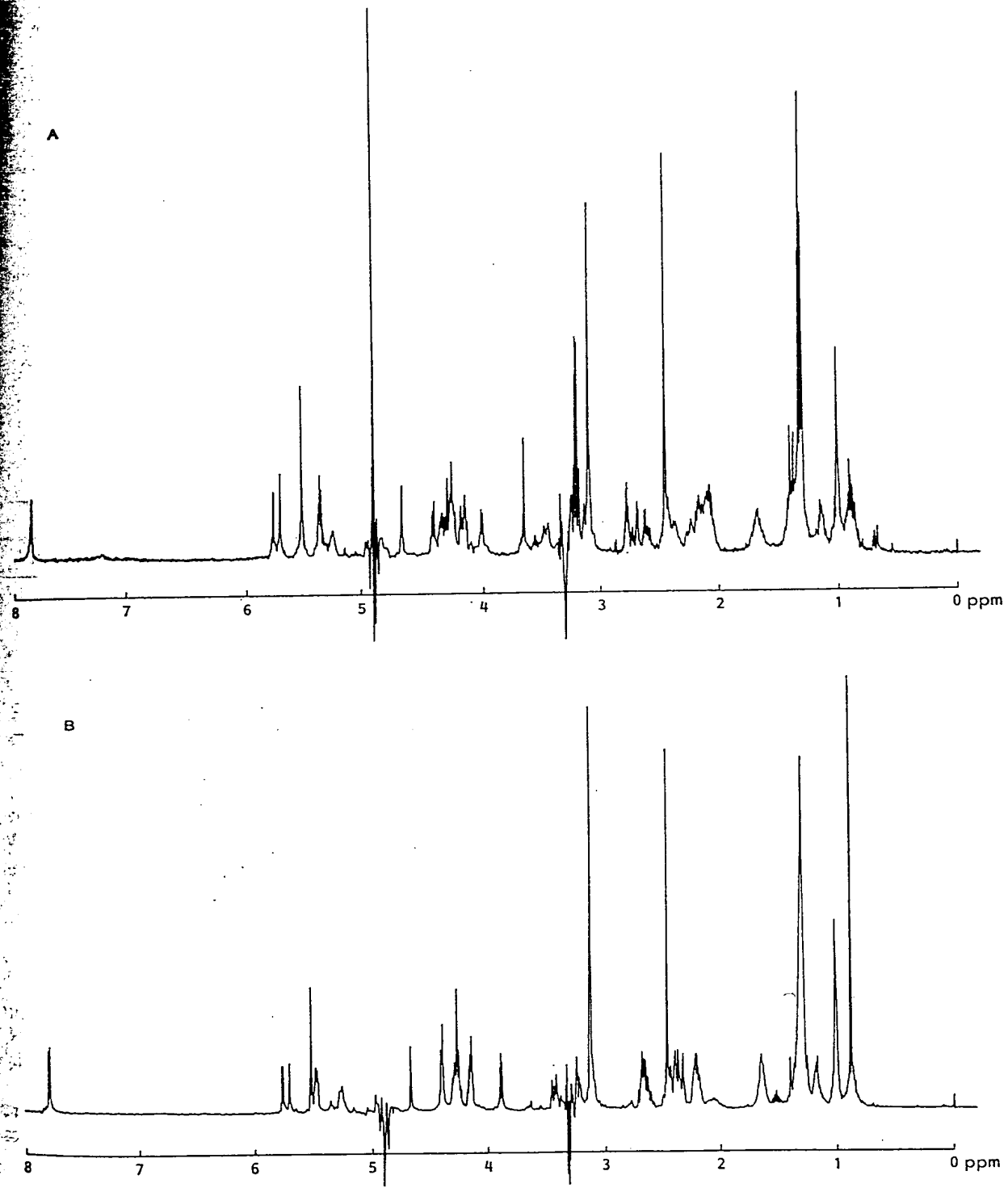


Fig. 3. (Continued).

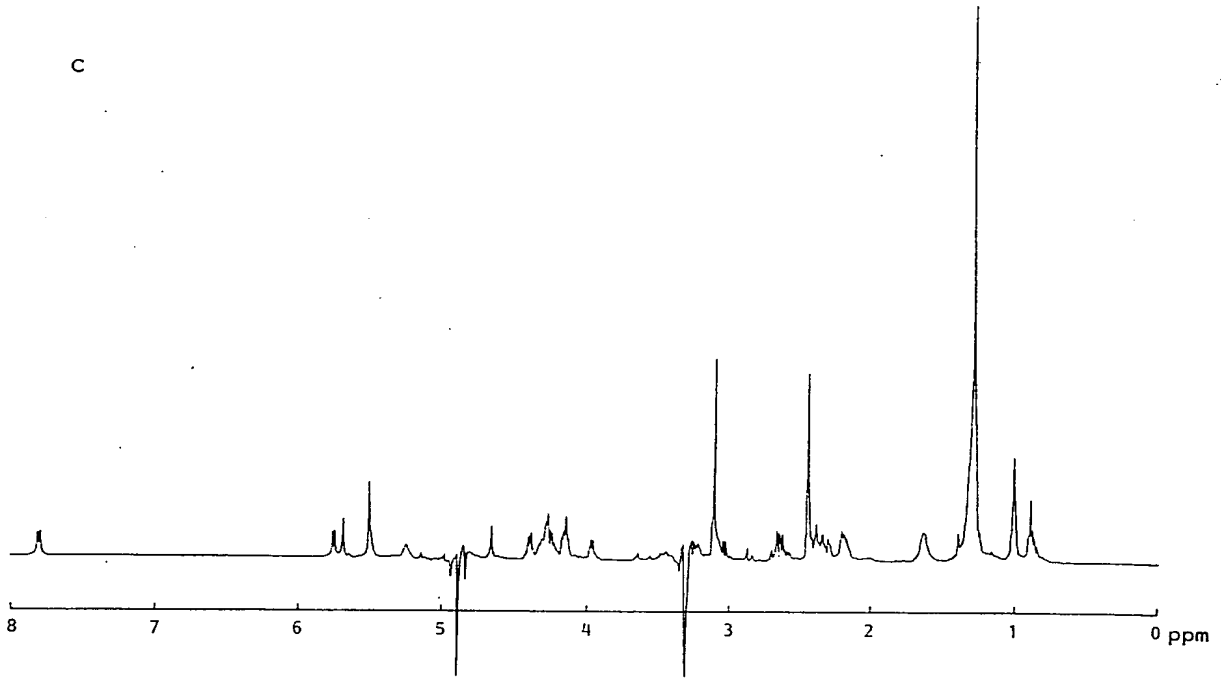


Table 1. Antibacterial activity of liposidomycins A, B and C.

Test organism	MIC (μg/ml)*		
	Liposidomycin A	Liposidomycin B	Liposidomycin C
Escherichia coli AB 1157	>100	>100	>100
E. coli BE 1186	50	100	100
Salmonella typhimurium TV 119	>100	>100	>100
Bacillus subtilis IFO 3513	100	100	100
Staphylococcus aureus IFO 12732	>100	>100	>100
Mycobacterium phlei IFO 3158	1.6	1.6	1.6

* Agar dilution method.

siderably different among the three components. The presence of uracil was confirmed by the isolation of uracil (EI-MS *m/z* 112, 69, 42) after hydrolysis of the complex by 3 N HCl.

Liposidomycins A, B and C strongly inhibited peptidoglycan synthetase prepared from *Escherichia coli* (ID_{50}, 0.03 μg/ml). This activity is three orders higher than that of tunicamycin and comparable to that of moenomycin. Contrary to the high *in vitro* activity, they showed a limited activity to some Gram-positive, Gram-negative and acid-fast bacteria (Table 1). Intravenous administration of 180 mg/kg of the complex caused no toxic response in mice. Mucopeptins[3~5], A38533[6] and FR-3383[7,8] are reported to contain uracil and sulfur, and are active against Gram-negative bacteria. However, UV spectra and optical rotations of liposidomycins are clearly different from these antibiotics.

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